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Perennial cover crop influences on soil C and N and maize productivity

Chumki Banik
Iowa State University, cbanik@iastate.edu

Cynthia A. Bartel
Iowa State University

David A. Laird
Iowa State University

Kenneth J. Moore
Iowa State University, kjmoore@iastate.edu

Andrew W. Lenssen
Iowa State University, alenssen@iastate.edu

Abstract
New management systems are needed that enhance the sustainability of crop residue harvesting for use as feedstock in the emerging biofuel industry. We investigated whether a novel perennial cover crop management system, designed to overcome yield drag, would enhance sustainability of maize (Zea mays L.) residue harvesting. Overall the perennial cover crop treatments (Kentucky bluegrass (Poa pratensis L.) (BG) and creeping red fescue (Festuca rubra L.) (RF)) increased the soil potential mineralizable N (8.5 %), decreased the loss of total soil organic C (10.1 %) and N (6.5 %) relative to the no-cover crop controls (with (RR) and without (RS) removal of crop residues). Respired CO2, measured during 28 day incubations, decreased in the following order: RF>RS≈BG>RR for both in-row and in-between-row samples implying high microbial activity under cover crop treatments. SPAD readings, growth stage, and end of season maize-stalk nitrate test results varied by site-year but were consistent with soil NH4+/NO3- dynamics. Results indicate that competition between the maize and perennial cover crops for water and N resources was weather dependent. Although previous research documented that the management system employed was able to overcome the yield drag associated with perennial cover crops, in our study maize yields for the perennial cover crop treatments were only one third the yields for the controls. Overall, we conclude that the perennial cover crop system is capable of enhancing the sustainability of maize residue harvesting, but more work is needed to overcome the yield drag which may be caused by perennial cover crops under some conditions.

Keywords
Maize, Perennial grass, Soil quality, Sustainable residue harvesting

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Perennial cover crop influences on soil C and N and maize productivity

Chumki Banik*, Cynthia A. Bartel, David A. Laird, Kenneth J. Moore, and Andrew W. Lenssen

Department of Agronomy, Iowa State University, Ames, IA 50011, USA

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*Corresponding author. Chumki Banik

3503 Agronomy Hall, Iowa State University, Ames, IA 50011, USA

Tel: +1-515-294-9233; Fax: +1-515-294-3163; email: cbanik@iastate.edu

ORCID, CB 0000-0002-6571-2493

ORCID, AWL 0000-0001-7468-671X

ABSTRACT

New management systems are needed that enhance the sustainability of crop residue harvesting for use as feedstock in the emerging biofuel industry. We investigated whether a novel perennial cover crop management system, designed to overcome yield drag, would enhance sustainability of maize (*Zea* mays L.) residue harvesting. Overall the perennial cover crop treatments (Kentucky bluegrass (*Poa pratensis* L.) (BG) and creeping red fescue (*Festuca rubra* L.) (RF)) increased the soil potential mineralizable N (8.5 %), decreased the loss of total soil organic C (10.1 %) and N (6.5 %) relative to the no-cover crop controls (with (RR) and without (RS) removal of crop residues). Respired CO_2, measured during 28 day incubations, decreased in the following order: RF>RS≈BG>RR for both in-row and in-between-row samples implying high microbial activity under cover crop treatments. SPAD readings, growth stage, and end of season maize-stalk nitrate test results varied by site-year but were consistent with soil NH_4^+/NO_3^- dynamics. Results indicate that competition between the maize and perennial cover crops for water and N resources was weather dependent. Although previous research documented that the management system employed was able to overcome the yield drag associated with perennial cover crops, in our study maize yields for the perennial cover crop treatments were only one third the yields for the controls. Overall, we conclude that the perennial cover crop system is capable of enhancing the sustainability of maize residue harvesting, but more work is needed to overcome the yield drag which may be caused by perennial cover crops under some conditions.

Key words Maize, Perennial grass, Soil quality, Sustainable residue harvesting

Abbreviations BG, Kentucky bluegrass; PMN, potential mineralizable N; RF, Creeping red fescue; RR, residue removal; RS, residue stays

1. Introduction

Maize (*Zea mays* L.) crop residue has the potential to be a major source of the biomass feedstock needed to meet the renewable fuel standard of 136 billion L of advanced biofuels as required under the Energy Independence and Security Act of 2007 (USDA 2011). Furthermore, there is competing demand for maize crop residue as feed and bedding for livestock (Kadam and McMillan 2003). In the short-term, removal of stover from continuous maize systems can increase grain yields in the upper Midwest (Karlen et al. 2011; Rogovska et al. 2016); however long-term harvesting of crop residues may reduce soil organic C levels and other measures of soil quality (Wilhelm et al. 2007; Laird and Chang 2013) and increase the susceptibility of soils to wind and water erosion. Therefore, several studies have suggested that only a limited portion of the aboveground biomass should be harvested to avoid long-term degradation of soil resources (Wilhelm et al. 2004; Wilhelm et al. 2007). The harvesting of crop residues is further challenging because sustainable harvest rates depend on yield, climate, topography, soil characteristics, and crop management practices (Wilhelm et al. 2004). Our research is motivated by the hypothesis that the use of perennial grass cover crops in continuous maize cropping systems will enhance soil quality and thereby allow a greater fraction of the above ground maize stover biomass to be sustainably harvested for biofuel production.

Perennial cover crop systems help to build soil organic matter, sequester C, increase soil microbial activity (Sainju et al. 2007) and below ground biomass, thereby reducing N leaching losses to ground water (Kramberger et al. 2009) relative to conventional and no-till cropping systems. Although there are many positive environmental outcomes, competitive perennial cover crops limit nutrient and water resources available to the primary crop, causing significant crop yield reductions, which can exceed 50% (Liedgens et al. 2004). For example, maize crops grown with perennial cover crops may suffer from N deficiency if not enough mineral N is present during the high demand period (Ranells and Wagger 1996; Kramberger et al. 2009). Hence, more research is needed to develop proper management practices and to select appropriate perennial cover crops to avoid yield losses.

One strategy to overcome the yield penalty is to suppress perennial cover crops before maize planting (Krueger et al. 2011; Alonso-Ayuso et al. 2014). Successful chemical or mechanical suppression of perennial cover crops reduces competition for moisture and nutrients between the cash crop and the cover crop. Indeed, good maize yields with perennial cover crops were achieved when at least 50% of a grass cover crop was chemically suppressed (Elkins et al. 1979). Another strategy is to use C3 grass species with inherent dormancy as a cover crop, therefore, minimizing the competition with maize. Wiggans et al. (2012) reported that strip tillage and early season chemical suppression of 'Troy' Kentucky bluegrass (*Poa pratensis* L.), a shallow-rooted pasture-type summer dormant C3 grass species, minimized competition between the perennial cover crop and resulted in high maize grain yields.

The overall goal of this study was to determine whether the perennial cover crop system developed by Wiggans et al. (2012) would enhance the sustainability of harvesting maize stover as a biomass feedstock for biofuel production by increasing or stabilizing the size of soil organic C and N pools relative to maize managed with no cover crop. The specific objectives of the current study were to understand the impact of perennial ground covers on: 1) soil N dynamics during the growing season, 2) soil C and N accretion/depletion, and 3) plant N uptake.

2. Materials and methods

2.1. Location, soil, treatment and management

A field study was conducted at the Iowa State University Agronomy and Agricultural Engineering Sorenson Research Farm (Sorenson; 42°0'N, 93°44'W), 11.9 km southeast of Boone, IA, from spring 2014 to fall 2016 and at the Iowa State University Northern Research Farm (Kanawha; 42°56'N, 93°47'"W), 0.5 km south of Kanawha, IA, from spring 2015 to fall 2016. The soils at the Sorenson site are predominantly very poorly drained Canisteo clay loams (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls), poorly drained Webster clay loams (fine-loamy, mixed, superactive, mesic Typic Endoaquolls), and moderately well drained Clarion loams (fine-loamy, mixed, superactive, mesic Typic Hapludolls) with 0-6% slopes; at the Kanawha site the soils are predominantly moderately well drained Clarion loams (fine-loamy, mixed, superactive, mesic Typic Hapludolls) with 2-6% slopes. The precipitation data were obtained from Iowa Environmental Mesonet, at sites located 3 km northwest of Sorenson (Ames-8-WSW Mesonet station) research farm and 17 km north of the Kanawha research farm (Britt Mesonet station).

The experiment was designed as a randomized complete block with four main treatments (Table 1), strip-tilled continuous maize production with residue removal grown with two cool season grass cover crop species, Kentucky bluegrass (BG) and creeping red fescue (RF), and two controls with continuous conventional tilled maize production with (RR) and without (RS) residue removal and two soil sampling protocols, in-row and in-between-row. All four main treatments were replicated three times at both sites (Sorenson and Kanawha). Baseline soil samples were collected during the establishment years, 2014 at Sorenson and 2015 at Kanawha. The baseline data is used to assess changes that occurred in soil properties that occurred as a result of the effects of the perennial cover crops during three site-years (2015 Sorenson, 2016 Sorenson, and 2016 Kanawha) after the perennial cover crops were established.

Each plot (111.4 m^2 = 9.14 m x 12.19 m) included twelve maize rows with 0.76 m inter-row spacings. The controls were conventionally managed with chisel plow tillage and either residue-stays (RS) or residue-removed (RR) treatments (Table 1). The perennial cover crop treatments were managed with maize residue removal each fall. Each row consisted of a 0.38 m spring tilled strip planted to maize and a 0.38 m interrow strip planted to either Kentucky bluegrass (BG) or creeping red fescue (*Festuca rubra* L.) (RF) perennial cover crops. The perennial grasses were seeded in spring 2013 at Sorenson but failed to establish an adequate cover in 2013 due to drought, hence the grasses were overseeded on May 6, 2014, to improve coverage. At Kanawha the perennial grasses were planted only once on 29 April 2015. More detailed on the grass establishment and other management practices used in this study is available in Bartel et al. (2017). A "population insensitive" (a maize variety adapted to high plant populations) maize variety (DKC57-75RIB, Monsanto, St. Louis, Missouri, USA) was planted on 30 May 2014, 22 May 2015, and 16 May 2016 at the Sorenson site, and on 1 April 2015 and 26 April 2016 at the Kanawha site.

At Sorenson on May 30th, 2014, April 28th, 2015, and May 4, 2016 P and K fertilizers were broadcasted at 90 kg P_2O_5 ha^{-1} as MAP (11-52-0) and 112 kg K_2O ha^{-1} as potash (0-0-60) on both the conventional and perennial cover crop plots. Actual N (191 kg-N ha^{-1}) was applied as S-coated urea (43-0-0-4; N-P-K-S) all three years. The N fertilizer was broadcast for controls (RR and RS) and banded near the maize row for the perennial cover crop systems (RF and BG) at planting for Sorenson in 2014 and 2015. For Sorenson 2016, a split application was used with 24 kg ha^{-1} N applied during maize planting and 168 kg ha^{-1} N applied as a side dress at the V5 maize growth stage. At Kanawha, P and K fertilizers, 211 kg P_2O_5 ha^{-1} and 291 kg ha^{-1} K_2O, were applied in Fall 2014 for the 2015 growing season, and 65 kg P_2O_5 ha^{-1} and 45 kg K_2O ha^{-1}

were applied on May 6, 2016, based on soil tests. Nitrogen fertilizer was applied at Kanawha using the same amount and same application protocol as described above for the Sorenson plots in 2014 and 2015.

Before maize seeding various herbicides, including, 2,4-D [2,4-dichlorophenoxyacetic acid], glyphosate [N-(phosphonomethyl)glycine], acetachlor [2-chloro- N-ethoxymethyl-N-(2-ethyl6-methylphenyl)acetamide] and atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], were used on the conventional plots at recommended rates (Bartel et al. 2017) to control grass and broad leaf weeds. Herbicides were also applied on the rows of the perennial cover crop plots using a banded sprayer with shields to keep the herbicides off the perennial grasses in the interrows. After the establishment year, the perennial cover crop plots were treated with paraquat (1,1'-Dimethyl-4,4'-bipyridinium dichloride) in early spring immediately after maize seeding to suppress the grasses during early season maize growth. Details of the management and agronomic practices for the establishment and growth of the perennial cover crops were reported elsewhere (Bartel et al. 2017).

2.2. Soil sampling and analysis

Before seeding the perennial cover crops in spring 2014 at the Sorenson site and in spring 2015 at the Kanawha site, six soil cores (0.0254 m diameter) were randomly taken from each treatment plot and composited by depth to produce 0-0.05 and 0.05-0.15 m composite soil samples. Establishment year post-harvest soil samples were also collected from both Sorenson (October 2014) and Kanawha (October 2015) sites using the same soil sampling protocol. The soil samples were placed in ziplock bags and refrigerated (below 4°C) until analysis. These soils were considered as baseline soil samples as the perennial cover crops were not yet fully established. In-season and post-harvest soil samples (described below) were collected in 2015 and 2016 and are compared with the baseline samples to assess the effects of the cover crop treatments on various soil quality indicators, including extractable NO_3-N and NH_4-N, soil moisture, soil pH, TC, TN, PMN, and respiration

Available N in post-harvest soil samples was determined by extracting 5g samples of field moist soil with 2M KCl using a 1:5 (mass:mass) soil to solution ratio. The mixture was shaken for 30 minutes and then filtered through Whatman No.42 filter paper and the filtrate was stored at -10°C before being analyzed for NO_3-N and NH_4-N using a colorimetric method (Hood-Nowotny et al. 2010). A method blank was also performed to account for any contamination, with each set of samples. After determining available N, the soil samples were air dried and sieved (<2 mm) before being further analyzed. Soil pH was determined with a glass electrode for samples equilibrated in Milli-Q water (18.2 MΩ·cm at 25 °C) at a 1:1 (mass:mass) ratio. Total C and N were analyzed using a C/N combustion analyzer (Vario Microcube, Elementar Analysensysteme GmbH, Langensebold, Germany) and Mehlich III extractable P and K were determined using ICP-OES (Mehlich 1984). The PMN was determined for samples from Sorenson in 2014 (after harvest), 2015 (spring, summer, fall and after harvest), and 2016 (after harvest) and for samples from Kanawha in 2015 before perennial cover crop establishment and in 2016 after harvest. To determine PMN, 20g of each soil sample was incubated at 30°C and 60% water filled pore space for 28 days. The difference between post and pre-incubation 2M KCl extractable inorganic N (sum of NO_3-N and NH_4-N) is reported as PMN (Drinkwater et al., 1996). During the incubation of 2015 Sorenson samples, another vial containing 5 mL of 1 M NaOH solution was placed in each of the incubation chambers (1L mason jars) with the soil to measure CO_2 flux. The NaOH vials were replaced with fresh NaOH vials on day(s) 1, 3, 6, 10, 15, 21 and 28. The amount of CO_2 captured in the vials was measured using back titration method (Anderson, 1994). Blanks without soil were always monitored the same way to account for background CO_2 levels during the incubation of the soils. Cumulative average CO_2 flux calculated for each 28 days incubation was used for statistical analysis.

The effects of the perennial cover crops on in-season soil N dynamics were assessed at Sorenson in both 2015 and 2016 and at Kanawha in 2016. To do so, 12 soil core samples were randomly collected from each treatment plot to depths 0-0.05 and 0.05-0.15 m at-least once per month from early spring (before maize planting) until late fall (after harvest). The composite samples were placed in zip lock bags by depth and refrigerated at 4°C. Within 24 hours of collection, the soil samples were homogenized and sieved (<2 mm), then sub-samples were taken to determine moisture and KCl-extractable inorganic N levels. The rest of the samples were air-dried and stored at 4°C. To determine moisture, soil subsamples were oven dried at 105°C until a constant weight was achieved. Soil inorganic N was determined using the method described earlier and the values corrected to a soil dry weight basis before data analysis. The soil sampling strategy varied during the growing season. Soils were randomly collected from each plot until the maize plants were at the V5 growth stage after which 12 plants per plot were randomly chosen and marked as sites for future soil sampling. Growth stage was assessed and soil plant analysis development (SPAD) index readings were measured using chlorophyll meter (SPAD 502, Spectrum Technologies, Brigend, Wales) for these 12 plants every other week. Soil samples were collected from within the maize row (in-row) and in between the maize row (in-between-row) from locations close to the marked plants.

2.3. Harvest, grain yield, and stalk nitrate test

At harvest, the four center rows of each plot were combined using a machine modified with an onboard weighing system for plot yield determination. About 90% of the maize stover was removed from each plot using John Deere 972 Flail Chopper (Deere & Company, Moline, IL) during late November or early December each site year. During harvest, the flail harvester cut the stalks 0.07 to 0.09 m above the ground to avoid any damage to the cover crops. During stalk sampling, any unharvested cobs found in the center 4 rows were manually harvested and added to the grain yields determined during mechanical harvesting. Maize cobs, stalks and grain were dried to a constant weight at 70°C and then weighed to determine aboveground dry matter yield for each plot. End of season maize stalk nitrate levels (Blackmer and Mallarino 1996) were determined using a 0.15-m stalk segment sampled between 0.15 and 0.30 m above the soil for the 12 marked maize plants. These stalk samples were dried at 60°C until constant weight, and then ground to pass a 1-mm sieve. Sub-samples of 1.5g of the ground stalks, along with appropriate blanks, were shaken with 100 mL 2M KCl for 15 minutes and filtered through Whatman 42 filter paper. The filtrate was stored at -10°C before being analyzed for stalk nitrates using a colorimetric method (Hood-Nowotny et al. 2010).

2.4. Experimental Design and Statistical Analysis

A weighted mean of nitrate, ammonium, moisture, PMN, TC and TN was calculated for all three site-years using the following equation:

$$X_{0\text{-}0.15m} = \{(\text{Value})_{0\text{-}0.05m} \text{ x } 1 + (\text{Value})_{0.05\text{-}015m} \text{ x } 2\}/3 \qquad [1]$$

To test significance of perennial cover crop effects, we analyzed data using PROC MIXED (SAS Institute 9.4) for the three post-establishment site-years choosing a model by the least value of the Akaike information criteria (AIC). Each year was analyzed independently for seasonal distribution of nitrate, ammonium, moisture, PMN (2015 Sorenson), and respiration (2015 Sorenson) using a three-way ANOVA model that included a repeated measure statement for time in the model. For SPAD index and Maturity, we used a two-way ANOVA model that included treatment and a repeated measure statement for time in the model. For total carbon change (TCchange), total nitrogen change (TNchange) and fall stalk nitrate levels we used a two-way ANOVA model that included treatment and row effects. Differences of least square means test were used for comparisons between treatments. All the analyses were performed using the P<0.05 level.

3. Results and discussion

3.1. Baseline soil properties

Prior to perennial cover crop establishment, soil properties varied slightly among the plots (Table 2), but no significant differences by treatment were present in the baseline soil properties for either location. However, there were differences in soil properties between locations. At Kanawha, soils are slightly more acidic (pH range 4.9 to 5.4) than soils at Sorenson (pH range 5.5 to 6.4). Soil organic C levels averaged 0.93% higher at Sorenson than Kanawha although C: N ratios were not significantly different. Extractable inorganic N was higher while Mehlich 3 extractable P and K were lower at Sorenson than Kanawha.

3.2. Precipitation

Average monthly precipitation (Fig. 1), as logged by Ames-8-WSW and Britt Mesonet stations, during the April through September maize growing season varied for each of the three site-years. Annual precipitation was higher than the 30 yr. average rainfall by 22% and 7% for 2015 and 2016 at Sorenson, respectively, and by 19% at Kanawha in 2016. However, June 2016 precipitation was 80% lower than the 30 yr. average for the same month at Sorenson and 35% lower at Kanawha. Drought conditions persisted at Sorenson into the third week of July 2016, after which heavy rains brought the July total above the 30 yr. average. Thus, although annual precipitation was above average, significant drought stress was evident during the 2016 growing season particularly at Sorenson.

3.3. Growing-season soil inorganic N levels

For the Sorenson 2015 site-year, soil inorganic N (NO_3-N plus NH_4-N) levels were elevated between Day 146 and Day 177 of 2015 in response to the fertilizer application (Fig. 2A). The three-way ANOVA (Table 3) shows parameters that were significantly influenced by soil inorganic N levels for the 0-0.15 m depth. Between Days 146 to 177 the in-row soil inorganic N levels were higher in the RF and BG plots than for the RR and RS plots, while in-between-row soil inorganic N levels tended to be higher for the RR and RS plots than the RF and BG plots. This difference reflects the fact that the N fertilizer was banded in RF and BG grass plots and broadcast in RR and RS plots. In general, soil NH_4-N levels were elevated between Days 146 to 154 period for the in-row samples but were below 10 mg kg^{-1} for all samples collected after Day 154. In-row soil NO_3-N levels did not show a response to fertilizer N application until Day 154, remained elevated for the Day 177 sampling, and then decreased to low levels (3-4 mg kg^{-1}) for all subsequent sampling dates. Soil NH_4-N and NO_3-N levels for the in-between-row samples showed similar but less pronounced response to N fertilization. The rapid depletion of NH_4-N and NO_3-N following fertilization at Sorenson in 2015 is attributed to both plant uptake during rapid vegetative growth and to leaching and/or denitrification due to the high amount of precipitation during June and July 2015 (Fig. 1).

The banded fertilizer application at Sorenson in 2016 increased in-row NH_4-N concentrations for all samplings between Day 173 and Day 223 but had only small effects on in-between-row NH_4-N concentrations (Fig. 2C). By contrast, the various treatments and sampling protocols (in-row vs in-between-row) had little effect on soil NO_3-N concentrations (Fig. 2D) at Sorenson in 2016. We attribute the high in-row NH_4-N and low NO_3-N concentrations for June and July at Sorensen in 2016 to the precipitation pattern. In 2016, near average rainfall occurred earlier in the growing season (April and May) followed by drought, which lasted until mid-July. Thus, we infer that low soil moisture levels in June and early July delayed nitrification, eliminated nitrate leaching, and put the maize under water stress.

For Sorensen 2016, treatment, row position, time, and treatment × time, time × row position, and treatment × time × row position interactions influenced NH_4-N concentration in 0-0.15 m soil depth (Table 3). These interactions are evident (Fig. 2C) in the timing of peak in-row soil NH_4-N concentrations, which occurred on Day 173 in the RR (45.3 mg kg^{-1}) and RS (53.0 mg kg^{-1}), on Day 187 in BG (62.0mg kg^{-1}) and on Day 204 in RR (80.1 mg kg^{-1}). Soil NO_3-N concentrations increased slightly with time but remained below 15 mg kg^{-1} through Day 187 and then increased up to 68% (BG-IR 9.01 mg kg^{-1} to 28.02 mg kg^{-1}) on Day 204 in response to precipitation. There was no difference between in-row and in-between-row soil NO_3-N concentrations on Day 204. For Sorenson in 2016, end of season extractable soil total inorganic N concentrations ranged from 8.2-11 mg kg^{-1}. Thus, the Sorenson 2016 data shows no evidence that the perennial cover crops competed with the maize crop for inorganic N, but does show that nitrification was substantially delayed due to drought.

For the third site-year, Kanawha 2016, soil NH_4-N and NO_3-N concentrations started to increase on Day 140 and stayed elevated through Day 200 in response to the fertilizer application

(Fig. 2E & 3F). Soil NH_4-N and NO_3-N concentrations varied by treatment, row position, time, and the time × row position interaction (Table 3). On Day 140 in-row soil NH_4-N levels in the RR and RS plots were higher (Fig. 2E) relative to the perennial cover crop plots (RF and BG), and in-row soil NH_4-N levels remained significantly greater until Day 154 in RF and Day 169 in BG. The in-between-row soil NH_4-N and NO_3-N concentrations showed a weak response to the banded fertilization application. The Kanawha 2016 soil inorganic N concentrations clearly indicate lower in-row NH_4-N levels for RF and BG plots relative to the RR plots from planting through mid-June. These results suggest that the perennial cover crops may have reduced the supply of inorganic N available to the maize crop at Kanawha in 2016.

The three site-years exhibited very different NH_4-N and NO_3-N dynamics following fertilizer N application. For Sorenson 2015, both NH_4-N and NO_3-N concentrations peaked on Day 154 then were rapidly depleted thereafter. For Sorenson 2016, NH_4-N concentrations remained high through July and there was only a small increase in NO_3-N before Day 204, suggesting delayed nitrification. For Kanawha 2016, soil NO_3-N levels started increasing on Day 140 and peaked between Days 154 and 169, indicating that nitrification occurred rapidly after fertilizer application. These differences in NH_4/NO_3 dynamics are attributed to different precipitation patterns, primarily during the month of June (Fig. 1). June precipitation at Sorenson in 2015 was well above the 30 yr average, which accelerated nitrification and NO_3^- leaching and/or denitrification. Severe drought during June and early July at Sorenson in 2016 delayed nitrification. Although June precipitation at Kanawha was below the 30 yr average, Kanawha 2016 had enough soil moisture for nitrification to proceed, but not enough precipitation to cause NO_3^- leaching.

3.4. Nitrogen fertility status of the maize crops

In this study, SPAD meter readings, growth stage assessments and the end of season maize-stalk nitrate tests were used to assess N fertility status of the maize for the three site-years. At Sorenson in 2015, the average SPAD reading for the RF was significantly lower than for the RR plots on Day 174 (Fig. S1A); thereafter there were no differences in SPAD readings by treatment. The growth stage data indicates that plants in RF (Fig. S2A) were slightly behind plants in other treatments on Day 195 but no significant treatment differences were observed. The end of season maize-stalk nitrate test values were similar and in the "low" category for all treatments (Table 4).

Sorenson 2016 SPAD reading were greater throughout the growing season than SPAD readings for Sorenson 2015, and SPAD readings in 2016 for the RR plots were greater than for other treatments during June and early July, but not thereafter. Pooled across the season SPAD readings were significantly impacted by treatment for Sorenson 2015 but not for Sorenson 2016 (Table 5). Growth stage results indicate that plants with perennial cover crops were slightly further developed on Day 210, but otherwise differences were negligible. The end of season maize-stalk nitrate test indicated that all treatments were similar and in the 'marginal' category for Sorenson 2016 (Table 4).

For Kanawha 2016, SPAD readings showed a consistent pattern throughout the season decreasing in the following order: RR>RS>RF>BG (Fig.S1C). Pooled across the season, however, SPAD readings were not influenced by treatment (Table 5). No growth stage differences were evident for Kanawha 2016; however, the end of season maize stalk nitrate test categories were 'marginal' for the RR plots and 'low' for all of the other plots with decreasing stalk nitrate levels in the following order: RR>RF=RS>BG (Table 4).

Overall, SPAD readings, growth stage, and end of season maize-stalk nitrate test results for each environment are consistent with soil NH_4/NO_3 dynamics. For Sorenson 2015, excessive June rainfall caused rapid nitrification and N loss to leaching and/or denitrification, resulting in severe N deficiencies impacting all plots. For Sorenson 2016, severe June drought delayed nitrification allowing high levels of NH_4 to remain in the soil for much of the growing season. The crops had adequate available N but crop growth was limited by water stress. Hence, the perennial cover crops had only a small effect on N availability and plant uptake of N at Sorenson in 2016. For Kanawha 2016, there was enough June rainfall to facilitate nitrification, but not enough to cause nitrate leaching. There was also enough moisture for maize growth at Kanawha 2016. The perennial cover crops apparently competed with the maize crop for N causing decreased NH_4-N supply during the early season and N deficiency in maize grown in the perennial cover crop plots. For all three site-years, the RR treatment had the greatest SPAD readings and the highest end of season stalk nitrate levels, suggesting that N immobilization during residue decomposition (RS) and immobilization and/or uptake of N by the perennial cover crops (RF and BG) reduced mineral N availability to the maize crop for these site years.

3.5. Cover crop impact on soil quality

Potentially mineralizable N is an index of soil quality that responds rapidly to soil management. Here we measured PMN from soil collected monthly through the 2015 growing season at Sorenson (Fig. 3A and B), and treatment significantly influenced PMN in 2015 (Table 6). The May 2015 soil samples collected shortly after N fertilizer application had lower PMN than samples collected at other times during the growing season. Other seasonal differences were not significant. The low PMN values for the May 2015 samples reflect high levels of inorganic N present in the soils at the time of sampling and losses of inorganic N to immobilization during the incubation. For months other than May, a relatively higher PMN for cover crop plots than non-cover crop plots was apparent in the PMN levels across the season and for both in-row and in-between-row samples. For the April, June, July, and October samplings, PMN generally decreased in the following order RF>RS≈BG>RR for both in-row and in-between-row samples. The treatment effect suggests that the removal of aboveground residues (RR) accelerates depletion of PMN from soils and that addition of fresh biomass residues either as maize stover (RS) or as perennial cover crop roots and residues (RF and BG) is needed to maintain PMN levels.

Respiration rate during aerobic incubations is another index of soil quality. Here we quantified respiration rates for samples collected monthly during the 2015 growing season at Sorenson (Figs. 3C and D). The 2015 Sorenson soil respiration rates were influenced by treatment (Table 6). The respired CO_2 decreased in the following order: RF>RS≈BG>RR for both the in-row and in-between-row samples. When the individual months were considered separately, respiration rates for the RF and RR differed in May, June, July and October for the in-between-row samples but not for the in-row samples. The lesser effect of the treatments on respiration for the in-row samples relative to the in-between-row samples is attributed to the strip tillage which was confined to perennial cover crop inter-rows. The respiration results suggest that the removal of crop residues (RR) accelerates depletion of labile soil organic C and that addition of biomass as maize stover (RS) or perennial cover crop residues and roots (RF and BG) is required to maintain levels of labile soil organic C.

Effects of the treatments on soil quality were further assessed by measuring post-harvest PMN the year that the treatments were imposed (baseline samples) and one year (Kanawha) and two years (Sorenson) after the treatments were imposed (Fig. 4A and B, respectively). No impact of the treatments on post-harvest PMN was evident the establishment year, 2014 for Sorenson and 2015 for Kanawha. Two years later, the Sorenson 2016 post-harvest soil samples had increased PMN relative to the establishment year for all four treatments and for both in-row and in-between-row samples, indicating an increase in soil organic N pool at the end of the study period. Differences in PMN among treatments were not observed for the 2016 post-harvest Sorenson samples (Fig. 4A). After only one year following perennial cover crop establishment, treatment effects on PMN were not observed for the post-harvest 2016 Kanawha samples.

The perennial cover crop treatments significantly affected the soil organic C and N over the study period. When BG and RF cover crops are considered separately, there were no significant changes in total soil C and N at the end of the study for either site (Table S1); however, the inter-row soils for the BG and RF treatments lost substantially less total N than inter-row RR and RS soils, respectively (Fig. S3). When the C and N data for plots with perennial cover crops (RF and BG) were pooled, the percent losses of soil organic C and N between 2014 and 2016 were decreased for the perennial cover crop systems at Sorenson (C: P=0.01 and N: P=0.02) (Figs. 5A). Although a similar trend was observed, the pooled effects of the perennial cover crop systems on changes in soil organic C and N levels was not significant (C: P=0.09 and N: P=0.2) for Kanawha in 2016 (Fig. 5B).

Soil moisture (g g^{-1}) measured across a growing season also can be a relative measure of soil quality. Assuming all plots receive the same rainfall, soil moisture levels integrate the combined effects of precipitation, infiltration/runoff, water holding capacity, evapotranspiration, and leaching. No significant effect of treatment on soil moisture was observed during the course of this study (Table 3; Fig. S4). At Sorenson 2016, there was relatively higher moisture in RS and RR plots compared with BG or RF plots on Day 173 (15.4, 14.8, 13.1 and 13.1% for RS, RR, RF, and BG, respectively), during the middle of the drought. Although not statistically significant this difference is consistent with competition between the cover crops and the maize crop for moisture under severe drought conditions. No indication of a cover crop effect on soil moisture was observed (Table 3) for the 2016 Sorenson and 2016 Kanawha site-years.

Nitrogen fertilizer management and weather effects for the three site-years are confounded in the present study. As such, the three site-years are not perfect replications; hence our comparisons across site-years are at a systems level.

3.6. Assessment of cover crop impact on crop yields

Yields of grain and stover have previously been reported (Bartel et al. 2017). Here, we discuss the impact of nitrogen, precipitation, and treatment interactions on maize yields for each site-year. Reductions in yield for BG (10.3 Mg ha^{-1}) and RF (8.3 Mg ha^{-1}) were 19 and 35%, respectively, relative to RR (12.8 Mg ha^{-1}) at Sorenson in 2015, implying that the difference in N placement (broadcast for control vs banded for cover crops) employed at Sorenson in 2015 was not able to overcome cover crop competition for N in a year with substantial losses from leaching and/or denitrification. Grain yields were 37% less for both BG (6.4 Mg ha^{-1}) and RF (6.5 Mg ha^{-1}) relative to RR (10.3 Mg ha^{-1}) at Sorenson in 2016. Soil tests (Fig. 2C), SPAD readings (Fig. S1B), and fall stalk nitrate test results (Table 4) indicate that an adequate supply of N was available regardless of treatment at Sorenson in 2016, however, low precipitation during the rapid vegetative growth stage put the maize plants under moisture stress and was the primary cause of yield reduction in 2016. Thus, the 2016 Sorenson results suggest that perennial cover crops can cause yield reductions by competing with maize for soil moisture. Maize grain yields were lower for plots with perennial cover crops 44% for BG (6.9 Mg ha^{-1}) and 29% for RF (8.8

Mg ha^{-1}) relative to the controls (12 Mg ha^{-1}) at Kanawha in 2016. Although minimal loss of N to leaching and/or denitrification occurred, there was apparently enough competition between the perennial cover crops and maize to cause shortages of N.

Overall, our grain yield results are generally consistent with previous literature on perennial cover crops. Eberlein et al. (2013) and Scott et al. (1987) have shown that low soil moisture due to low precipitation increases maize-cover crop competition and adversely impacts maize grain yields. The detrimental impact of water stress caused by drought on grain yield of continuous maize also has been reported in other studies (Varvel, 1994; Linden et al. 2000; Wilhelm and Wortmann 2004; Gentry et al. 2013). Our results from Sorenson in 2016 suggest that using strip tillage and selection of the partially dormant C3 cool season grasses for use as a cover crop was not enough to overcome competition for moisture during a drought year. Our results for Sorenson 2015, a year with excess precipitation that depleted soil inorganic N, indicate that perennial cover crops competed with the maize for inorganic N and aggravated crop N shortages.

Results for Kanawha in 2016, a year with adequate but not excessive soil moisture, indicated that the perennial cover crops competed with maize for N resources causing N deficiency when no deficiency was observed for the RR control. Thus our study supports the previous observations that the availability of N to maize during rapid vegetative growth, when there is a high demand for N, is crucial and that competition between the grass species cover and maize for N during this stage can reduce grain yields (Kramberger et al. 2009). In contrast with our results, Wiggans et al. (2012) observed comparable and in one case greater maize grain yields using a similar management system, a BG cover crop with strip tillage and spring chemical burn-down of the cover crop. Key differences between our study and Wiggans et al.

(2012) are: 1) Wiggans et al. used an older variety of Kentucky bluegrass that had a greater tendency for summer dormancy than the variety used in this study, 2) they achieved better early season control of competition between the perennial cover crop and maize than was achieved in our study, and 3) they used point injection of a 32% UAN solution for late spring sidedress N fertilization applications while we banded sulfur coated urea in either single pre-plant or split applications. Wiggans et al. (2012) reported fall stalk nitrate levels averaging 599, 1522, and 774 mg NO_3–N kg^{-1} for their three site-years. These fall stalk nitrate levels are all in the 'optimum' range indicating adequate N fertility through the growing season, whereas our fall stalk nitrate levels were 'marginal' (Sorenson 2016) or 'low' (Sorenson 2015 and Kanawha 2016) for the perennial cover crop systems indicating that our N fertilization strategy did not supply sufficient N fertility for the maize crops.

The current study provided evidence that the perennial cover crop treatments were rapidly building labile soil organic C and N as evidenced by increasing PMN and soil respiration rates (Fig. 3). These results indicate rapid improvement in soil quality during the short one and two years that the perennial cover crops were in place. Building labile soil organic matter inherently involves N immobilization (Ranells and Wagger 1996), which may have contributed to the decreased N availability for maize in our study. The new soil organic C and N, which accumulates under a perennial cover crop, is labile as evidenced by the increase in PMN and respiration rates as observed here. Thus, it is reasonable to assume a transition period following the seeding of a perennial cover crop during which substantial amounts of inorganic N will be immobilized, and after which a new steady state will be achieved with greater gross N mineralization during the growing season. During the transition period, relatively greater rates of N fertilization may be required to ensure an adequate supply of N for both the maize crop and immobilization of N in new labile soil organic matter, which is accumulating under the perennial cover crop. After the new steady state has been achieved, it may be possible to decrease N fertilization rates for perennial cover crop systems as the turnover of labile soil organic matter should provide substantial in-season N fertility. The present study provides evidence to support N immobilization during a transition phase, but our study was not conducted enough years to test the ‘new steady state’ hypothesis.

Numerous studies have reported that maize grain yields are suppressed in continuous maize systems relative to maize-soybean rotations in the upper US Midwest and that grain yields in continuous maize systems can be increased by harvesting the previous year’s maize stover (Karlen et al. 2011; Rogovska et al. 2016). Several factors appear to contribute to the yield suppressing effect of maize stover including: colder, wetter soils in the spring, harboring of insects and pathogens in the stover, allelopathy, and N immobilization as the previous year’s residue decompose (Yakle and Cruse 1984; Singh et al. 2010; Gentry et al. 2013; Rogovska et al. 2014). Hence, the harvesting of maize stover for bioenergy production provides a short-term economic opportunity for farmers, who may profit both from the sale of the residue and from a boost in maize grain yields in continuous maize systems with stover removal, along with a long-term risk of soil quality degradation due to the depletion of labile soil organic matter (Lal 1997; Wilhelm et al. 2004; Laird and Chang 2013).

5.0 Conclusions

The results of the present study provide evidence that perennial cover crops can rapidly increase soil quality in continuous maize systems even when all above ground maize residues are harvested. This suggests that perennial cover crops have the potential to enhance the sustainability of crop residue harvesting for bioenergy production and increase the amount of residue that can be sustainably harvested. However, competition between maize and perennial cover crops for water and N resources may occur depending on weather. Hence, further work is needed to optimize N fertility management and minimize early season competition between the perennial cover crop and the maize crop, to consistently obtain high yields in perennial cover crop-maize systems.

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References

Alonso-Ayuso, M., Gabriel, J.L., Quemada, M., 2014. The kill date as a management tool for cover cropping success. PLoS ONE 9 (10) e109587. doi:10:1371/journal.pone.0109587.

Anderson, J.P.E., 1994. Soil respiration. In: Page, A.L., Miller, R.H., Keeney, D.R., editors, Methods of Soil Analysis Part 2: Chemical and microbiological properties. ASA-SSSA Inc., Madison, WI. p. 831-866.

Bartel, C.A., Banik, C., Lenssen, A.W., Moore, K.J., Laird, D., Archontoulis, S.A., Lamkey, K.R., 2017. Establishment of perennial groundcovers for maize-based bioenergy production systems. Agron. J. 109, 822-835.

Blackmer, A.M., Mallarino, A.P., 1996. Cornstalk testing to evaluate nitrogen management. PM 1584. University Extension, Iowa State University. https://store.extension.iastate.edu/Topic/Crops/Soil-Management-and-Fertility.

Drinkwater, L.E., Cambardella, C.A., Reed, J.D., Rice, C.W., 1996. Potentially mineralizable nitrogen as a property of biologically active soil nitrogen. In: Doran, J.W., Jones, A.J. (Eds.), Methods for Assessing Soil Quality. SSSA Spec. Publ. 49. SSSA, Madison, WI, pp. 217–229.

Eberlein, C.V., Sheaffer, C.C., Oliveira, V.F., 2013. Corn growth and yield in an alfalfa living mulch system. J. Prod. Agric. 5, 332-339.

Elkins, D.M., Vandeventer, J.W., Kapusta, G., Anderson, M.R., 1979. No-tillage maize production in chemically suppressed grass sod. Agron. J. 71, 101-105.

Gentry, L.F., Ruffo, M.L., Below, F.E., 2013. Idetifying factors controlling the continuous corn yield penalty. Agron. J. 105, 295-303.

Hood-Nowotny, R., Umana, N.H.-N., Inselbacher, E., Wanek, P.O.-L.W., 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. Soil Sci. Soc. Am. J. 74, 1018-1027.

Kadam, K.L., McMillan, J.D., 2003. Availability of corn stover as a sustainable feedstock for bioethanol production. Bioresour. Technol. 88, 17-25.

Karlen, D.L., Varvel, G.E., Johnson, J.M.F., Baker, J.M., Osborne, S.L., Novak, J.M., Adler, P.R., Roth, G.W., Birrell, S.J., 2011. Monitoring soil quality to assess the sustainability of harvesting corn stover. Agon. J. 103, 288-295.

Kramberger, B., Gselman, A., Janzekovic, M., Kaligaric, M., Bracko, B., 2009. Effects of cover crops on soil mineral nitrogen and on the yield and nitrogen content of maize. Eur. J. Agron. 31, 103-109.

Krueger, E.S., Ochsner, T.E., Porter, P.M., Baker, J.M., 2011. Winter rye cover crop management influences on soil water, soil nitrate, and corn development. Agron. J. 103, 316-323.

Laird, D.A., Chang, C.W., 2013. Long-term impacts of residue harvesting on soil quality. Soil Tillage Res. 134, 33-40.

Lal, R., 1997. Long-term tillage and maize monoculture effects on a tropical Alfisol in western Nigeria. II. Soil chemical properties Soil Tillage Res. 42, 161-174.

Liedgens, M., Frossard, E., Richner, W., 2004. Interactions of maize and Italian ryegrass in a living mulch system: (2) nitrogen and water dynamics. Plant Soil 259, 243-258.

Linden, D.R., Clapp, C.E., Dowdy, R.H., 2000. Long-term corn grain and stover yields as a function of tillage and residue removal in east central Minnesota. Soil Tillage Res. 56, 3-4.

Mehlich, A., 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Comm. Soil Sci. Plant Anal. 15, 1409-1416.

Ranells, N.N., Wagger, M.G., 1996. Nitrogen release from grass and legume cover crop monocultures and bicultures. Agron. J. 88, 777-782.

Rogovska, N., Laird, D.A., Karlen, D.L., 2016. Corn and soil response to biochar application and stover harvest. Field Crops Res. 187, 96-106.

Rogovska, N., Laird, D.A., Rathke, S.J., Karlen, D.L., 2014. Biochar impact on Midwestern Mollisols and maize nutrient availability. Geoderma 230, 340-347.

Sainju, U.M., Schomberg, H.H., Singh, B.P., Whitehead, W.F., Tillman, P.G., Lachnicht-Wayers, S.L., 2007. Cover crop effect on soil carbon fractions under conservation tillage cotton. Soil Tillage Res. 96, 205-218.

Scott, T.W., Pleassant, J.M., Burt, R.F., Otis, D.J., 1987. Contributions of ground cover, dry matter, and nitrogen from intercrops and cover crops in a corn polyculture system. Agron. J. 79, 792-798.

Singh, N.B., Amit, S., Deepmala, S., 2010. Autotoxicity of maize and its mitigation by plant growth promoting rhizobacterium Paenibacillus polymyxa. Allelopathy J. 25, 195-204.

USDA, 2011. U.S. Billion-ton update: Biomass supply for a bioenergy and bioproducts industry. Perlack, R.D, Stockes, B.J., (Leads). ORNL/TM-2011/224. United States Department of Energy, Oak Ridge National Lab., Oak Ridge, TN. p. 227.

Varvel, G.E., 1994. Monoculture and rotation system effects on precipitation use efficiency of corn. Agron. J. 86, 204-208.

Wiggans, D.R., Singer, J.W., Moore, K.J., Lamkey, K.R., 2012. Response of continuous maize with stover removal to living mulches. Agron. J. 104, 917-925.

Wilhelm, W., Johnson, J.M.F., Hatfield, J.L., Voorhees, W.B., Linden, D.R., 2004. Crop and soil productivity response to corn residue removal: A literature review. Agron. J. 96, 1-17.

Wilhelm, W., Wortmann, C.S., 2004. Tillage and rotation interactions for corn and soybean grain yield as affected by precipitation and air temperature. Agron. J. 96, 425-432.

Wilhelm, W.W., Johnson, J.M.F., Karlen, D.L., Lightle, D.T., 2007. Corn stover to sustain soil organic carbon further constrains biomass supply. Agron. J. 99, 1665-1667.

Yakle, G.A., Cruse, R.M., 1984. Effects of fresh and decomposing corn plant residue extracts on corn seedling development. Soil Sci. Soc. Am. J. 48, 1143-1146.

Figure legends

Fig. 1. Monthly (April-September) precipitation (2015-2016) at Ames-8WSW mesonet station, which is located approximately 3km NW from Sorenson research farm site, and at the Britt mesonet station (2016), which is located approximately 17 km N of the Kanawha Research farm site, and 30 year mean monthly precipitation for these locations.

Fig. 2. Seasonal distribution of nitrate-N and ammonium-N concentration in soils (0-0.15 m) during the maize growing season for Sorenson 2015 (A: NH_4-N; B: NO_3-N), Sorenson 2016 (C: NH_4-N; D: NO_3-N), and Kanawha 2016 (E: NH_4-N; F: NO_3-N). Here IR represents in-row and IBR represents in-between-row. Data points are averages for three plot replications, significant differences are discussed in the text.

Fig. 3. (A and B) Potential mineralizable N (PMN) and (C and D) respiration rates for in-row (IR) and in-between-row (IBR) soil samples (0-0.15 m) collected monthly from Sorenson during the 2015 growing season. Error bars represent standard errors of means.

Fig. 4. Potential mineralizable N (PMN) for soil samples (0-0.15 m) collected from (A) Sorenson and (B) Kanawha. Significant differences ($P<0.05$) between establishment year samples (Sorenson Fall 2014 and Kanawha Fall 2015) and final year in-row (IR) and in-between-row (IBR) samples (Sorenson Fall 2016 and Kanawha Fall 2016) are indicated (*). No significant within year treatment effects were observed.

Fig. 5. Treatment effects on changes in soil (0-0.15 m) organic C and N between establishment year samples collected after harvest (Sorenson June 2014 and Kanawha June 2015) and samples collected after harvest the final year of the study, (A) Sorenson 2016 and (B) Kanawha 2016. Pooled (BG and RF) cover crop treatments (CC) and pooled no-cover crop (RR and RS) treatments (NCC) are also compared. Error bars represent standard errors of means.

*Table 1.*Summery of treatments and management practices used for each site year (establishment years not shown).

Site-year	Treatments			
	Residue removed (RR)	Blue grass (BG)	Residue Stays (RS)	Red creeping fescue (RF)
Sorenson 2015	No cover crop Conventional tillage Population insensitive Residue removed Conventioanl herbicide applications Single application of broadcast urea N (191 kg-N ha^{-1})	Bluegrass cover crop Zone tillage Population insensitive Residue removed Conventional herbicides banded over maize, paraquat over cover crop Single application of banded urea N (191 kg-N ha^{-1})	No cover crop Conventional tillage Population insensitive Residue stays Conventioanl herbicide applications Single application of broadcast urea N (191 kg-N ha^{-1})	Red Fescue cover crop Zone tillage Population insensitive Residue removed Conventional herbicides banded over maize, paraquat over cover crop Single application of banded urea N (191 kg-N ha^{-1})
Sorenson 2016	No cover crop Conventional tillage Population insensitive Residue removed Conventioanl herbicide applications Split application of broadcast urea N (191 kg-N ha^{-1})	Bluegrass cover crop Zone tillage Population insensitive Residue removed Conventional herbicides banded over maize, paraquat over cover crop Split application of banded urea N (191 kg-N ha^{-1})	No cover crop Conventional tillage Population insensitive Residue stays Conventioanl herbicide applications Split application of broadcast urea N (191 kg-N ha^{-1})	Red Fescue cover crop Zone tillage Population insensitive Residue removed Conventional herbicides banded over maize, paraquat over cover crop Split application of banded urea N (191 kg-N ha^{-1})
Kanawha 2016	No cover crop Conventional tillage Population insensitive Residue removed Conventioanl herbicide applications Single application of broadcast urea N (191 kg-N ha^{-1})	Bluegrass cover crop Zone tillage Population insensitive Residue removed Conventional herbicides banded over maize, paraquat over cover crop Single application of banded urea N (191 kg-N ha^{-1})	No cover crop Conventional tillage Population insensitive Residue stays Conventioanl herbicide applications Single application of broadcast urea N (191 kg-N ha^{-1})	Red Fescue cover crop Zone tillage Population insensitive Residue removed Conventional herbicides banded over maize, paraquat over cover crop Single application of banded urea N (191 kg-N ha^{-1})

Table 2. Characteristics of baseline soils collected prior to cover crop establishment.

Site	Treatments	Depth (m)	pH (1:1 soil:water)	TC (g kg^{-1})	TN (g kg^{-1})	KCl extractable NO_3^- - N + NH_4^+-N (mg kg^{-1})	Mehlich3 P (mg kg^{-1})	Mehlich3 K (mg kg^{-1})
Sorenson 2014	Blue Grass	0-0.05	5.6± 0.09*	28.8± 7.1	2.5± 0.4	20.24± 3.15	36.84±5.03	174.53± 24.11
		0.05-0.15	5.5± 0.13	27.9± 9.1	2.4± 0.6	19.18± 4.79	18.29± 1.80	112.85± 7.80
	Red Fescue	0-0.05	6.4 ± 0.43	34.1± 12.3	3.0± 0.8	21.66± 4.05	38.02± 2.28	189.51± 1.69
		0.05-0.15	6.4± 0.48	34.4± 13.4	3.0± 0.9	18.70± 3.84	15.59± 2.02	109.80± 2.96
	Residue Removed	0-0.05	5.6 ± 0.06	33.8± 6.0	2.8± 0.4	17.84± 3.47	35.43± 2.15	151.51± 11.46
		0.05-0.15	5.6± 0.16	33.9± 6.6	2.8± 0.5	19.11± 6.00	15.06± 2.59	88.79± 8.44
	Residue Stay	0-0.05	5.9± 0.22	30.3± 10.2	2.7± 0.7	21.88± 4.84	36.31± 4.49	166.25± 8.33
		0.05-0.15	6.0± 0.26	30.9± 11.9	2.7± 0.9	20.00± 4.61	18.46± 5.78	101.25± 19.06
Kanawha 2015	Blue Grass	0-0.05	5.1± 0.48	22.9± 1.4	2.1± 0.1	17.36± 0.50	68.52± 3.55	378.05± 44.3
		0.05-0.15	4.9± 0.36	21.6± 2.4	2.0± 0.17	13.02± 1.73	35.87± 3.74	110.30± 15.18
	Red Fescue	0-0.05	5.4± 0.31	20.3± 3.9	1.9± 0.3	17.22± 4.00	82.52± 20.27	410.43± 73.60
		0.05-0.15	5.1± 0.32	18.5 ± 2.9	1.8± 0.3	12.56± 1.28	31.96± 10.7	113.18± 22.82
	Residue Removed	0-0.05	5.3± 0.29	21.4± 1.1	2.0± 0.14	16.80± 0.18	64.93± 10.89	369.02± 26.73
		0.05-0.15	5.1± 0.28	20.5± 1.5	1.9± 0.1	12.20± 0.73	31.52± 8.90	93.53± 14.72
	Residue Stay	0-0.05	5.1± 0.31	21.0± 1.4	1.9± 0.06	16.35± 1.47	68.81± 3.08	370.20± 8.78
		0.05-0.15	4.9± 0.24	19.2 ± 2.1	1.8± 0.26	13.94± 0.60	27.30± 8.73	101.74± 24.52

TC, total carbon; TN, total nitrogen.

*standard errors of mean for n=3.

Table 3. Output from a three-way ANOVA model of 0 to 0.15-m depth soil ammonium, nitrate and moisture concentrations as an interactive function of treatment, row and time. Separate models were run for each site year.

Site - year	Effect	Ammonium P value	Nitrate	Moisture
Sorenson 2015	Block	0.04	<0.0001	<0.0001
	Treatment	0.12	0.43	0.08
	Row	0.002	0.013	0.72
	Treatment*row	0.07	0.011	0.87
	Time	<0.001	<0.0001	<0.0001
	Treatment*time	0.12	<0.0001	0.85
	Time*row	0.003	<0.0001	<0.0001
	Treatment*time*row	0.53	<0.0001	0.27
Sorenson 2016	Block	0.95	<0.0001	0.01
	Treatment	0.05	0.0003	0.614
	Row	<0.0001	0.23	0.342
	Treatment*row	0.09	0.92	0.011
	Time	<0.0001	<0.0001	<0.0001
	Treatment*time	0.0002	0.08	<0.0001
	Time*row	<0.0001	0.29	0.013
	Treatment*time*row	0.0003	0.81	0.46
Kanawha 2016	Block	0.81	0.007	0.019
	Treatment	0.02	0.0017	0.38
	Row	<0.0001	<0.0001	0.47
	Treatment*row	0.22	0.115	0.28
	Time	<0.0001	<0.0001	<0.0001
	Treatment*time	0.09	0.005	0.87
	Time*row	<0.0001	<0.0001	0.528
	Treatment*time*row	0.517	0.31	0.75

Table 4. Fall maize stalk NO_3-N levels (Blackmer and Mallarino, 1996). Data are averages of 12 plants per plot and three plots per treatment. Different letters indicate significant differences (P<0.05) between treatments within a site-year

Treatments	Sorenson 2015	Sorenson 2016	Kanawha 2016
	------------mg NO_3^- -N kg^{-1}-----------		
BG	16.1 (a)	252.1 (a)	41.3 (b)
RF	13.8 (a)	473.1 (a)	180.9 (b)
RR	81.6 (a)	511.9 (a)	611.3 (a)
RS	22.9 (a)	510.5 (a)	171.6 (b)

Low = less than 250 mg kg^{-1}Marginal = 250 to 700 mg kg^{-1}
Optimal = 700 to 2000 mg kg^{-1}
Excess = greater than 2000 mg kg^{-1}

Table 5. Output from a two-way ANOVA model of plant maturity and SPAD index as an interactive function of treatment and time.

Site-year	Effect	Maturity P value	SPAD P value
Sorenson 2015	Treatment	0.01	0.02
	time	<0.0001	<0.0001
	Treatment*time	0.01	0.004
Sorenson 2016	Treatment	0.053	0.16
	time	<0.0001	<0.0001
	Treatment*time	0.017	0.18
Kanawha 2016	Treatment	0.14	0.07
	time	<0.0001	<0.0001
	Treatment*time	<0.0001	0.91

Table 6. Output from a three-way ANOVA model for soil potential mineralizable N and respiration (CO_2 released) as an interactive function of treatment, row and time.

Effect	Potential mineralizable N P value	Respirational CO_2 P value
Block	<0.0001	<0.0001
Treatment	0.03	<0.0001
Row	0.34	0.86
Treatment*row	0.333	0.01
Time	<0.0001	<0.001
Treatment*time	0.13	0.25
Time*row	0.051	0.01
Treatment*time*row	0.62	0.24

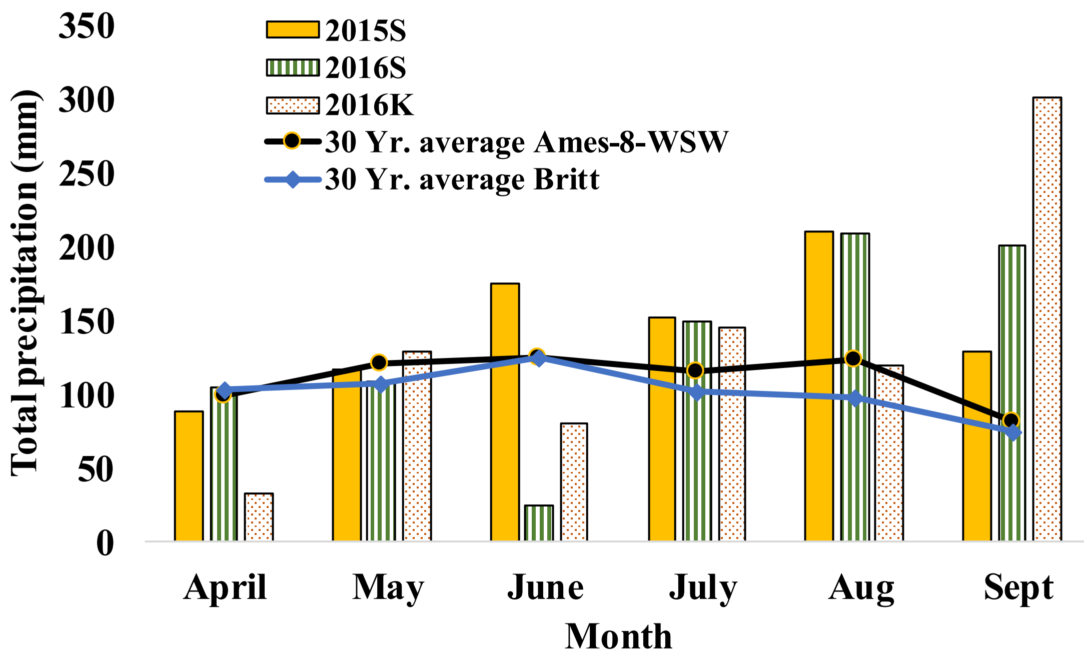

Figure 1. Monthly (April-September) precipitation (2015-2016) at Ames-8WSW mesonet station, which is located approximately 3km NW from Sorenson research farm site (S), and at the Britt mesonet station (2016), which is located approximately 17 km N of the Kanawha Research farm site (K), and 30 year mean monthly precipitation for these locations.

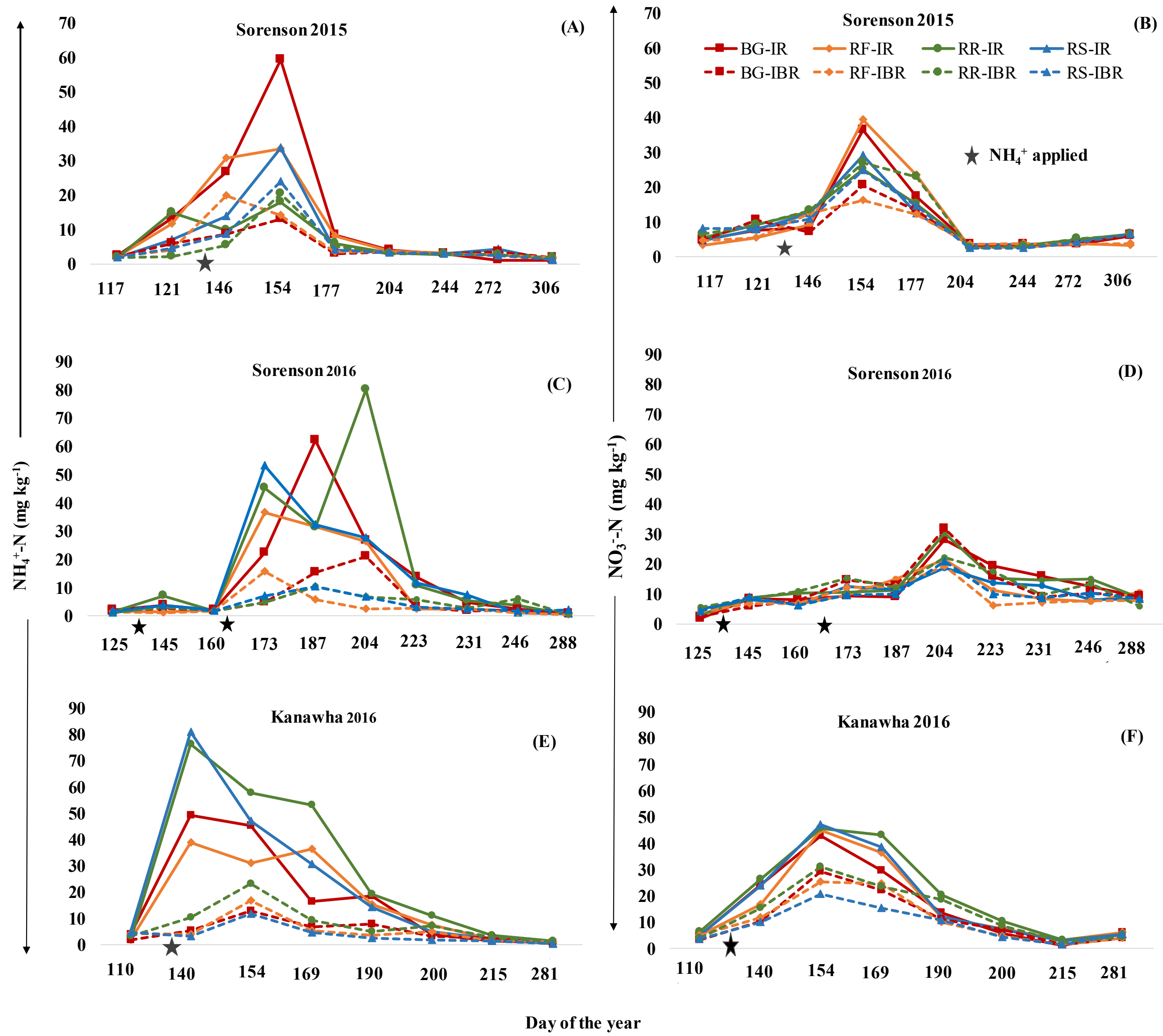

Figure 2: Seasonal distribution of nitrate-N and ammonium-N concentration in soils (0-0.15 m) during the maize growing season for Sorenson 2015 (A: NH_4-N; B: NO_3-N), Sorenson 2016 (C: NH_4-N; D: NO_3-N), and Kanawha 2016 (E: NH_4-N; F: NO_3-N). Here IR represents in-row and IBR represents in-between-row. Data points are averages for three plot replications, significant differences are discussed in the text.

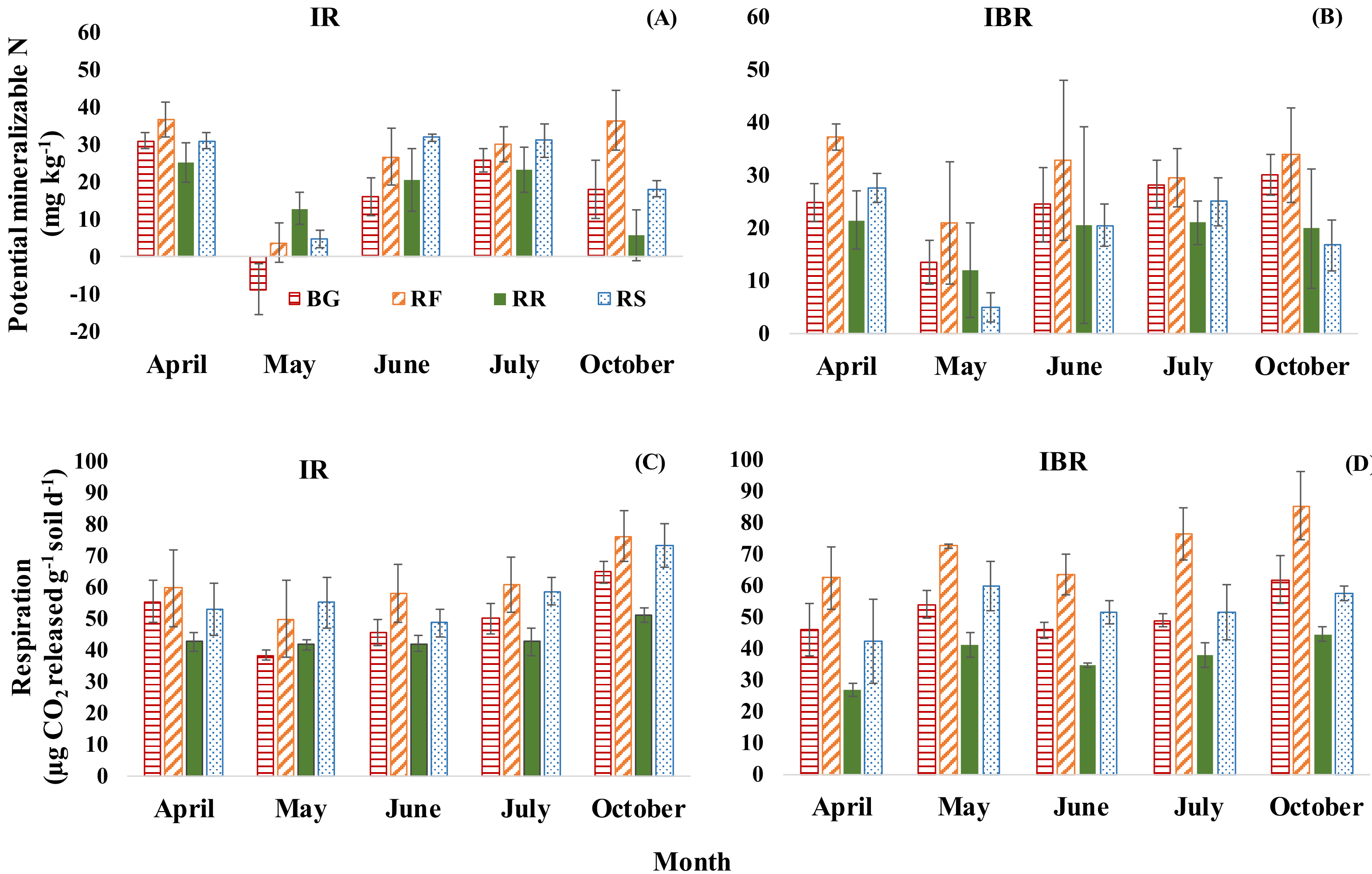

Figure 3: (A and B) Potential mineralizable N (PMN) and (C and D) respiration rates for in-row (IR) and in-between-row (IBR) soil samples (0-0.15 m) collected monthly from Sorenson during the 2015 growing season. Error bars represent standard errors of means.

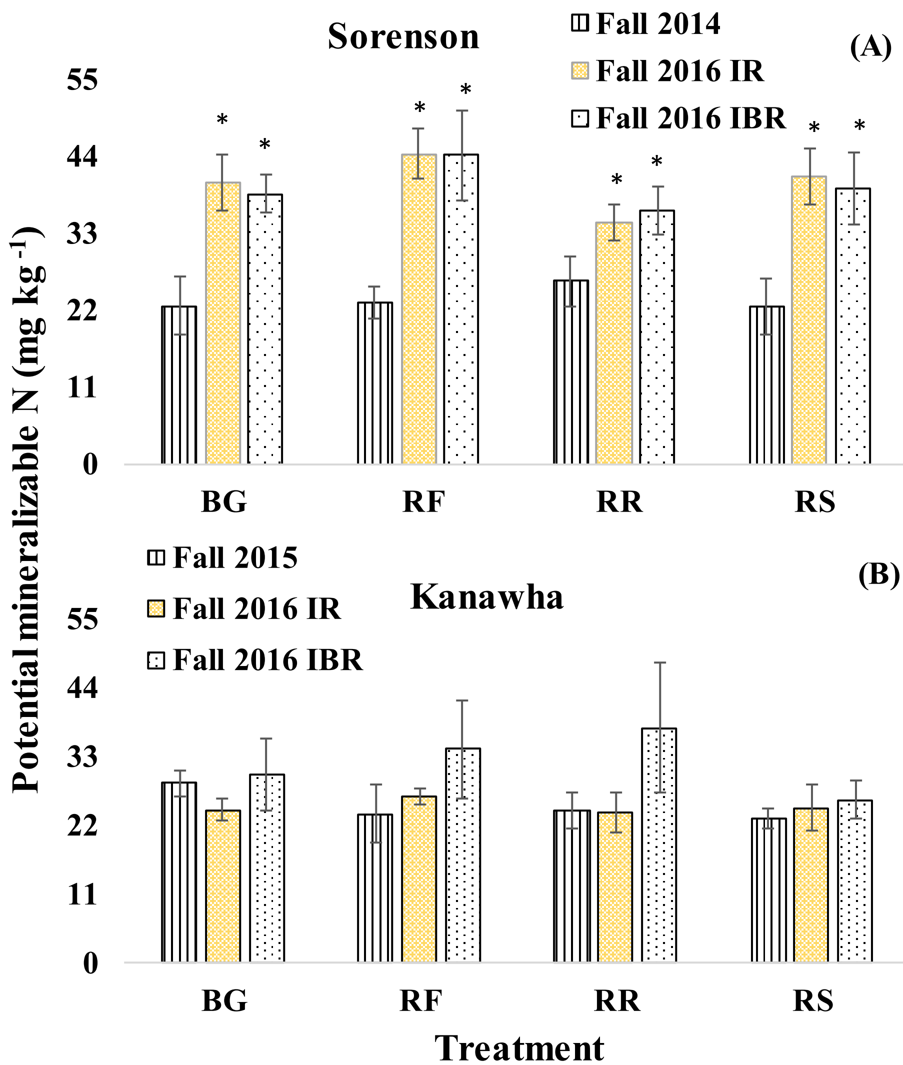

Figure 4: Potential mineralizable N (PMN) for soil samples (0-0.15 cm) collected from (A) Sorenson and (B) Kanawha. Significant differences (P<0.05) between establishment year samples (Sorenson Fall 2014 and Kanawha Fall 2015) and final year in-row (IR) and in-between-row (IBR) samples (Sorenson Fall 2016 and Kanawha Fall 2016) are indicated (*). No significant within year treatment effects were observed.

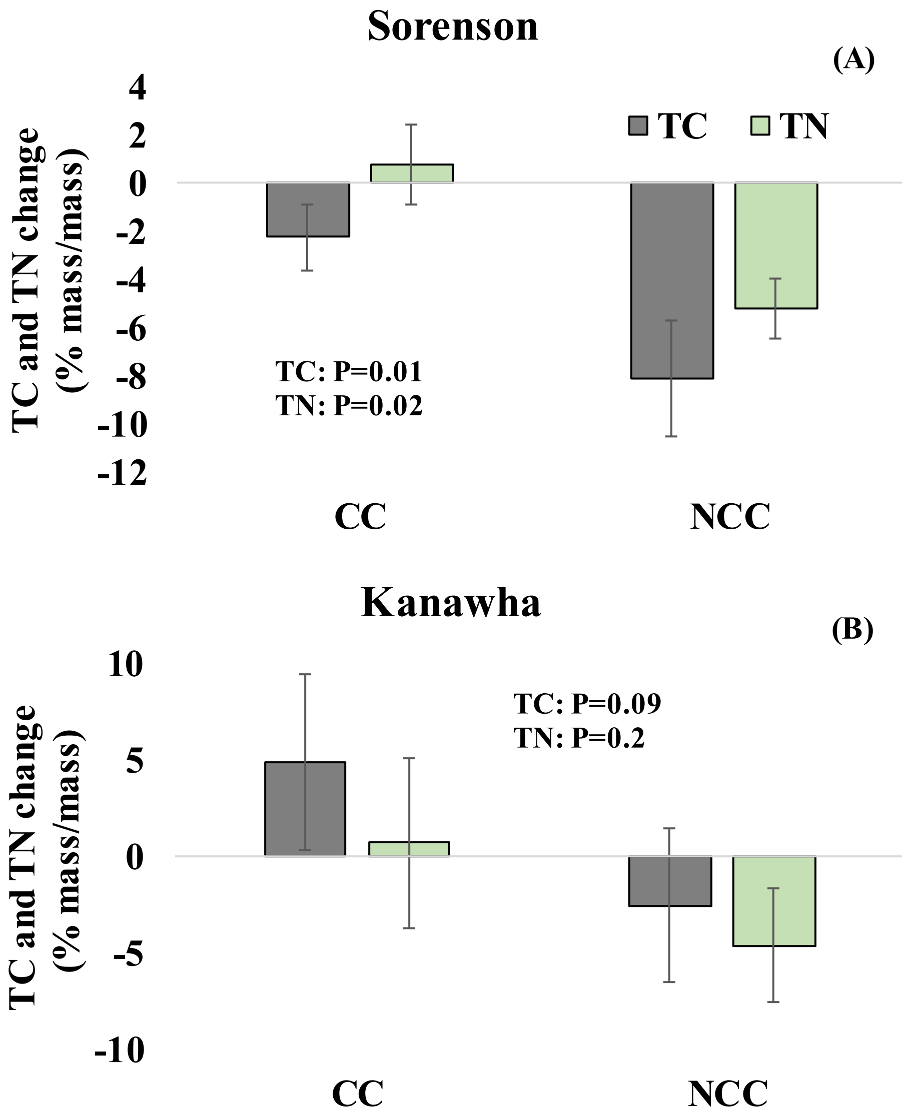

Figure 5: Treatment effects on changes in soil (0-0.15 cm) organic C and N between establishment year samples collected after harvest (Sorenson June 2014 and Kanawha June 2015) and samples collected after harvest the final year of the study, (A) Sorenson 2016 and (B) Kanawha 2016. Pooled (BG and RF) cover crop treatments (CC) and pooled no-cover crop (RR and RS) treatments (NCC) are also compared. Error bars represent standard errors of means.

Supplementary information

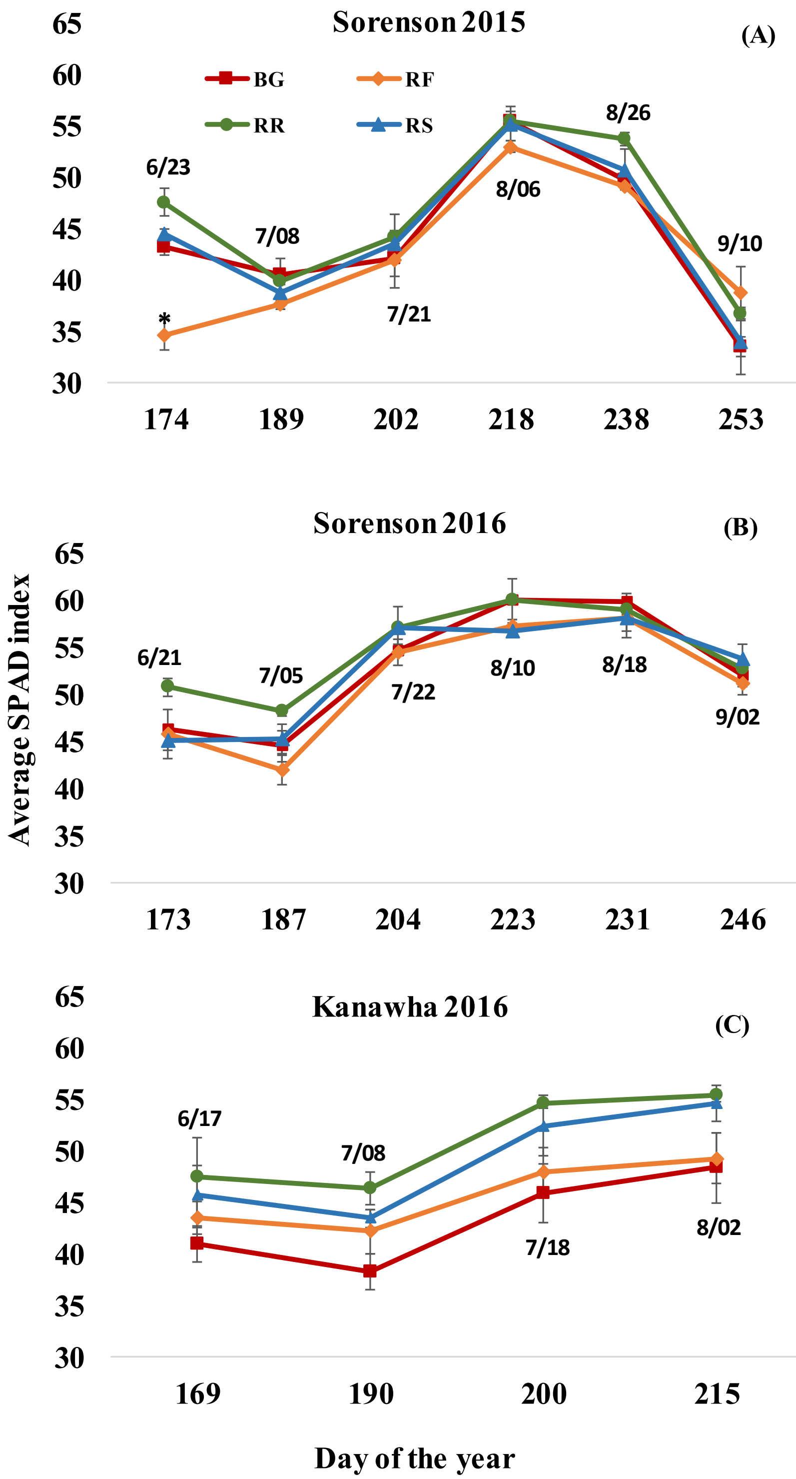

Figure S1: Average SPAD readings for (A) Sorenson 2015, (B) Sorenson 2016, and (C) Kanawha 2016 site-years. SPAD readings are averages for 12 plants per plot.

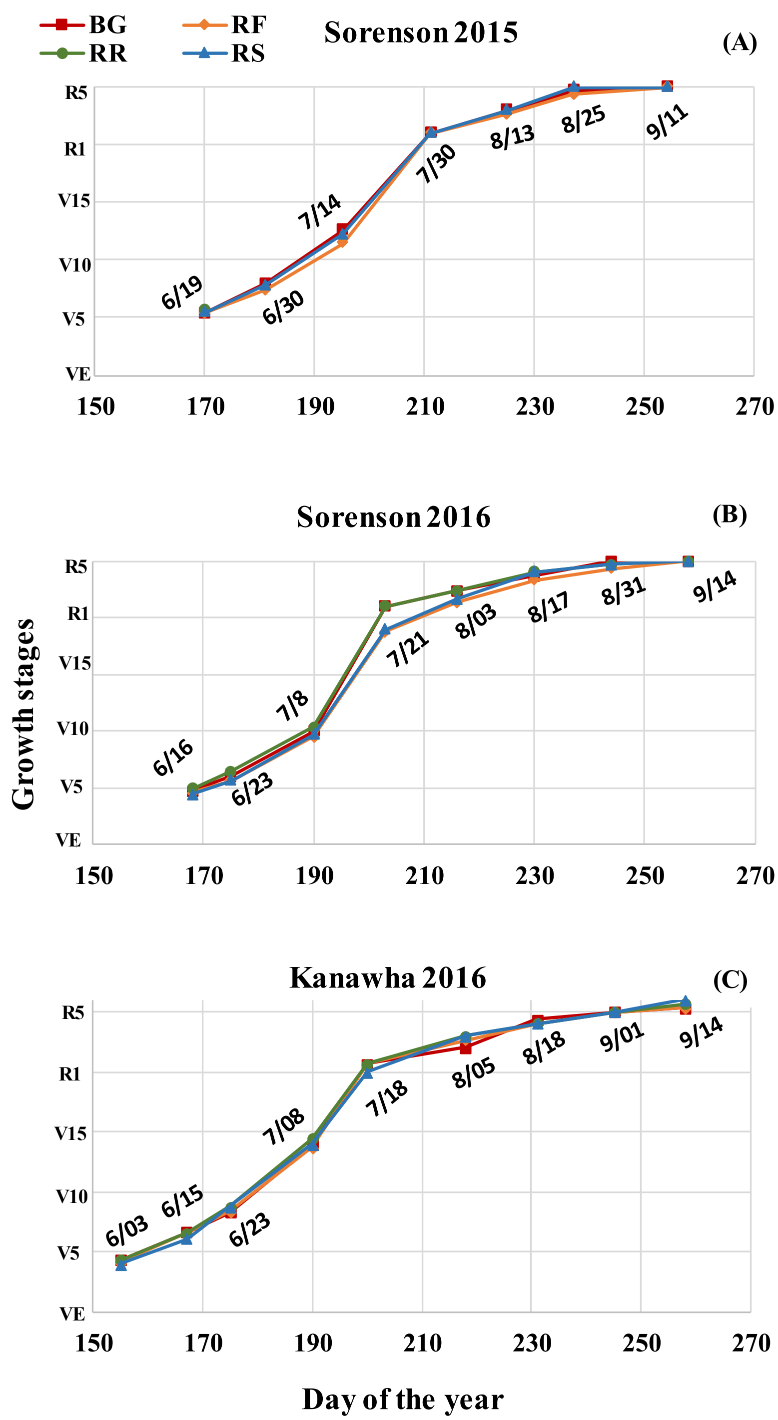

Figure S2: Maize growth stages for the (A) Sorenson 2015, (B) Sorenson 2016, and (C) Kanawha 2016 site-years. Data are averages of 12 plants per plot and three plots per treatment.

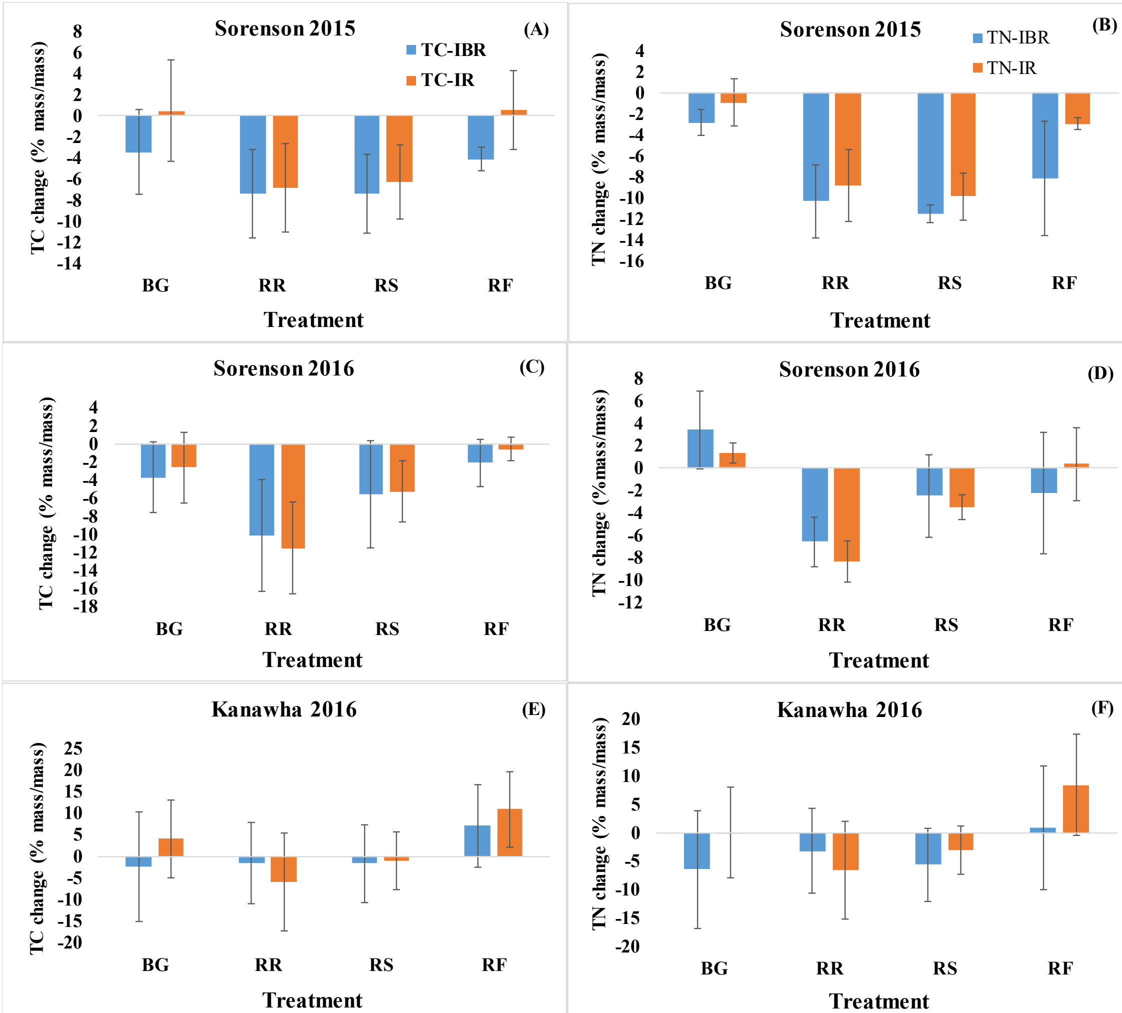

Figure S3: Treatment effects on changes in soil (0-0.15 m) organic C and N from baseline samples (Sorenson June 2014 and Kanawha June 2015) relative to soil samples collected after harvest; (A) Sorenson 2015 TC, (B) Sorenson 2015 TN (C) Sorenson 2016 TC, (D) Sorenson 2016 TN, and (E) Kanawha 2016 TC, (F) Kanawha 2016 TN. Error bars represent standard errors of means.

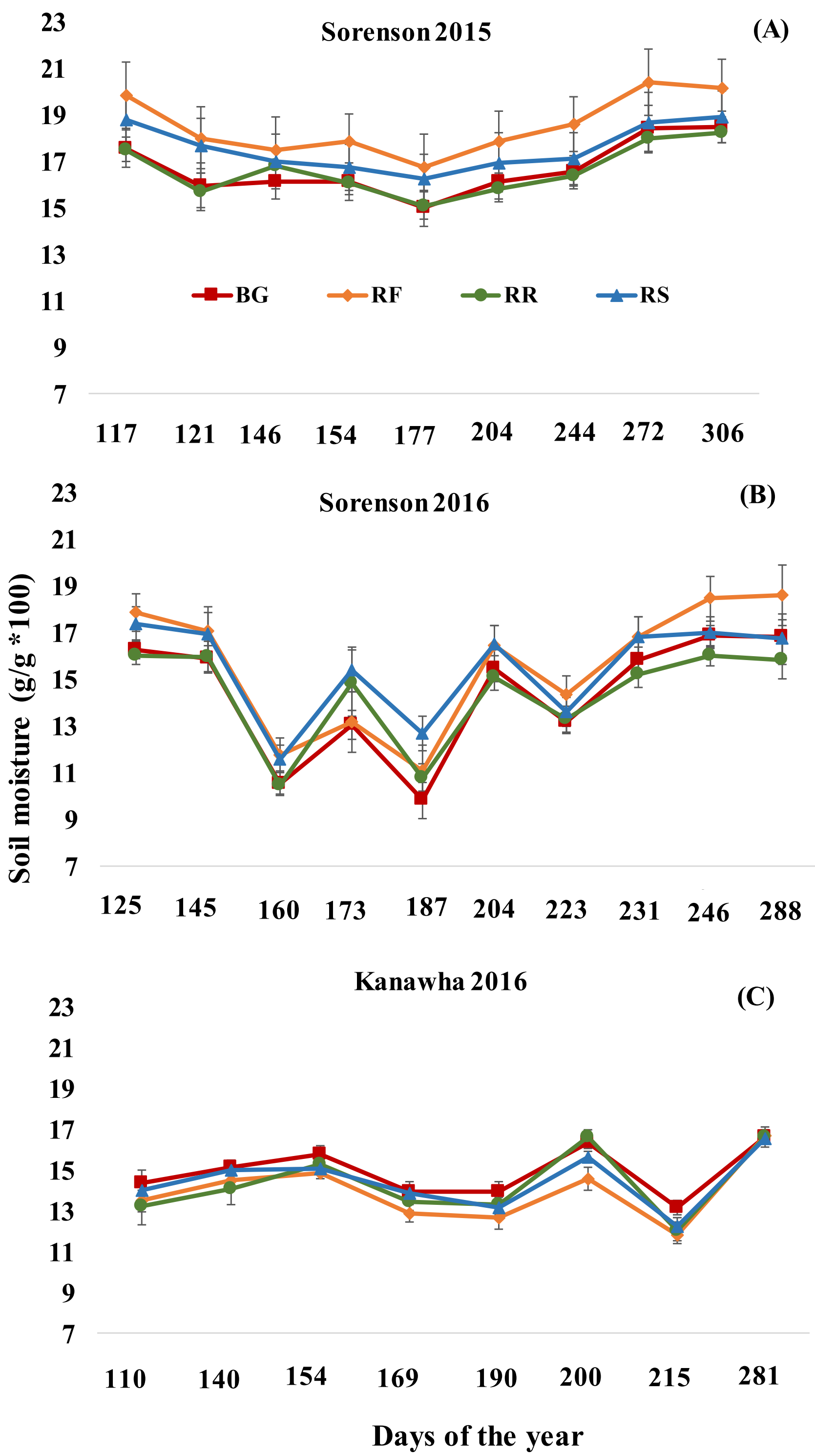

Figure S4: Gravimetric moisture content for soils (0-0.15 m) from (A) Sorenson 2015, (B) Sorenson 2016, and (C) Kanawha 2016 plots measured during the maize growing season. Average moisture content for composite samples collected from three plots per treatment. Error bars represent standard errors of means.

Table S1: Output from a two way ANOVA model (s) for Sorenson and Kanawha site respectively. Here TCchange and TNchange from baseline samples for the final year of the experiment analyzed as an interactive function of treatment, and row.

Site-year	**Effect**	**TC change**	**TN change**
		P	P
Sorenson 2016	Treatment	0.2	0.19
	Row	0.52	1.0
	Treatment*row	0.7	0.8
Kanawha 2016	Treatment	0.81	0.84
	Row	0.25	0.02
	Treatment*row	0.13	0.07